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K/DOQI-recommended intact PTH levels do not prevent low-turnover bone disease in hemodialysis patients

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The guidelines proposed by the Kidney Disease Outcomes Quality Initiative (K/DOQI) suggested that intact parathyroid hormone (iPTH) should be maintained in a target range between 150 and 300 pg ml^{-1} for patients with stage 5 chronic kidney disease. Our study sought to verify the effectiveness of that range in preventing bone remodeling problems in hemodialysis patients. We measured serum ionized calcium and phosphorus while iPTH was measured by a second-generation assay. Transiliac bone biopsies were performed at the onset of the study and after completing 1 year follow-up. The PTH levels decreased within the target range in about one-fourth of the patients at baseline and at the end of the study. The bone biopsies of two-thirds of the patients were classified as showing low turnover and a one-fourth showed high turnover, the remainder having normal turnover. In the group achieving the target levels of iPTH 88% had low turnover. Intact PTH levels less than 150 pg ml^{-1} for identifying low turnover and greater than 300 pg ml^{-1} for high turnover presented a positive predictive value of 83 and 62%, respectively. Our study suggests that the iPTH target recommended by the K/DOQI guidelines was associated with a high incidence of low-turnover bone disease, suggesting that other biochemical markers may be required to accurately measure bone-remodeling status in hemodialysis patients.

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Renal osteodystrophy (ROD) is an alteration of bone morphology that occurs in patients with chronic kidney disease (CKD).[1] ROD comprises a wide spectrum of manifestation that includes high-turnover states, such as predominant hyperparathyroid bone disease (PHBD) and mixed uremic osteodystrophy (MUO), and low-turnover states, such as osteomalacia and adynamic bone disease (ABD). Our understanding of this complex disorder has evolved during the last decades. Nevertheless, its evaluation continues to be a challenge for the practicing nephrologists.

Bone biopsy is required for the definitive diagnosis of ROD.[1] The invasive nature of the procedure as well as its cost and overall complexity has withdrawn it from the clinical practice. Hence, serum markers of bone turnover have been used to evaluate bone turnover in CKD patients. Measurement of intact parathyroid hormone (iPTH) has long been considered the principal biochemical marker for diagnosis and monitoring therapy of ROD.[2] In 1986, Sherrard and co-workers[3] suggested that iPTH could be a good predictor of osteitis fibrosa in patients undergoing maintenance hemodialysis. Further studies have demonstrated the direct relationship between iPTH and bone histomorphometric parameters, mainly bone formation rate.[4–7] Consequently, iPTH has taken the leading position among the non-invasive tools for assessing ROD. Recently, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF K/DOQI) Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease have provided guidance on the use of iPTH to evaluate ROD. A target range of plasma iPTH for stage 5 CKD patients has been suggested to be between 150 and 300 pg ml^{-1}.[8] Although this evidence-based target has been backed on well-designed studies,[4–7] they were performed more than a decade ago, when the management of ROD was quite different from now.

This study is part of a randomized controlled trial comparing the effects of sevelamer with those of calcium acetate on bone metabolism of chronic hemodialysis patients. Despite not being the primary purpose of the study, it was

used to examine the effectiveness of the K/DOQI-proposed target range of iPTH in preventing bone remodeling derangements in stage 5 CKD patients.

RESULTS

A total of 101 patients were enrolled in the study. Ninety-seven patients provided adequate bone samples for histomorphometric analysis. Table 1 shows the demographic and biochemical characteristics of the patients at baseline. Results for the entire population and for each histological group, namely low-turnover bone disease (LT), high-turnover bone disease (HT), and NL (normal histology) are shown. The mean age of the entire population was 49.5 ± 13.1 years, most of them were white (58%) and men (67%). They were on hemodialysis for 36.4 ± 24.8 months. An association between LT and being of white race and a trend toward an association with older age ($P = 0.07$) and diabetes ($P = 0.07$) was noticed. There was no difference regarding bone findings between the group allocated to sevelamer and that allocated to calcium acetate treatment. LT patients also presented significantly lower serum iPTH levels, and less time on hemodialysis. The percentage of patients on 2.5 mequiv. l^{-1} dialyzate calcium concentration was significantly higher in LT as compared to HT. LT patients have been taking a lower dose of calcitriol.

The histological diagnoses of the first bone biopsies are shown in Figure 1. LT was the most frequent histological finding ($n = 58$, 60%; Figure 1a). HT was observed in 36 (37%) patients. The majority of the LT was comprised of ABD (95%), whereas MUO and PHBD comprised 67 and 33% of the HT group, respectively (data not shown on the figure). Three patients had normal bone histology. Figure 1b shows the distribution of these histological findings according to the K/DOQI range of iPTH. The most prevalent bone disorders observed below the lower and above the upper limit of the iPTH range (150–300 pg ml^{-1}) were LT ($n = 29$, 83%) and HT ($n = 25$, 62.5%), respectively. LT was the most common finding ($n = 14$, 64%) in the ideal range of iPTH.

Sixty-four patients (aged 47.5 ± 12.7 years, 66% men, 62.5% white) concluded the follow-up. The causes of dropout were kidney transplantation ($n = 12$), death ($n = 9$), referral for peritoneal dialysis ($n = 5$), refusal for second bone biopsy ($n = 5$), and parathyroidectomy ($n = 2$). These patients did not differ from the ones who completed the study regarding age (51.6 ± 13.5 vs 47.5 ± 12.7 years), time on dialysis (34.4 ± 24.6 vs 37.5 ± 25.1 months), serum phosphorus (5.9 ± 1.2 vs 5.8 ± 1.0 mg per 100 ml), serum ionized calcium (1.28 ± 0.09 vs 1.28 ± 0.07 mmol l^{-1}), serum iPTH (361.6 ± 337.9 vs 420.4 ± 307.5 pg ml^{-1}), and the histological type of bone lesion.

Figure 2 shows the bone histological findings from first bone biopsy and how they changed from one category to another after 1 year. Bone histology did not change in approximately 60% of the patients with LT (23/37) and HT (15/26). No patient turned to normal bone histology. The second bone biopsy could not be performed in 21 and in 10 patients with LT and HT, respectively. The unique patient with normal bone histology who ended the follow-up remained in this category. Concerning ROD management during the study, there was no difference in calcitriol dose, using 2.5 mequiv. l^{-1} dialysate calcium concentration, type of phosphate binder, and having been on deferoxamine treatment, between patients who changed and did not change their bone histological lesion to another category.

Figure 3 depicts the results of the second bone biopsy after 1-year follow-up. LT was still the most prevalent bone disorder ($n = 34$, 53%), followed by HT ($n = 29$, 45.5%; Figure 3a). LT group was comprised mainly of ABD (97%), whereas PHBD and MUO comprised 62 and 38%, respectively, of the HT group (data not shown on the figure). One

Table 1 | Demographic and biochemical characteristics for the entire population and for each histological group at baseline

	Entire population (*n*=97)	LT (*n*=58)	HT (*n*=36)	NL (*n*=3)
Age (years)	49.5 ± 13.1	50 ± 13.6	45.4 ± 12.2	54.3 ± 4.0
Gender (% male)	65	69	61	33.3
Race (% white)	58	67*	42	66.6
Time on hemodialysis (months)	36.4 ± 24.8	30.1 ± 20.8*	48.2 ± 26.9	36.7 ± 28.9
Diabetes mellitus (%)	16.5	21	8	0
Dialyzate [Ca^{2+}]=2.5 mequiv. l^{-1} (%)	41	52*	25	33.3
Calcitrol dosage (μg day^{-1})[a]	0.04 ± 0.15	0.01 ± 0.05*	0.09 ± 0.23	0
Phosphate binder (%)[b]				
Calcium acetate	48	50	41.7	66.7
Sevelamer	52	50	58.3	33.3
Serum ionized calcium (mmol l^{-1})	1.28 ± 0.1	1.28 ± 0.1	1.26 ± 0.07	1.36 ± 0.24
Serum phosphorus (mg per 100 ml)	5.4 ± 1.5	5.4 ± 1.6	5.4 ± 1.2	4.9 ± 2.9
Serum iPTH (pg ml^{-1})	391.6 ± 389.9	266.4 ± 316.2*	613.8 ± 414.6	145 ± 72.8
Al.S/BS > 25% (%)	41	46.5	36	0

Al.S/BS, aluminum bone surface; HT, high-turnover bone disease; iPTH, intact parathyroid hormone; LT, low-turnover bone disease; NL, normal histology.
*$P < 0.05$ vs HT.
[a]Mean dose of calcitriol at baseline.
[b]Refers to the percentage of patients allocated to each group of phosphate binder at the beginning of the study.

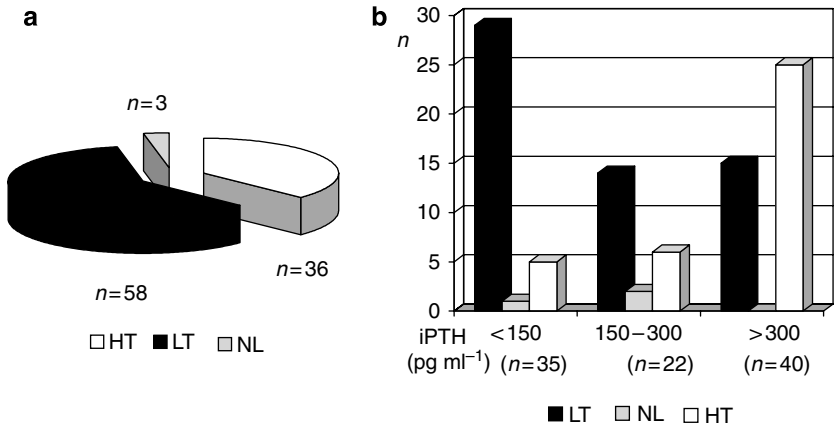

Figure 1 | Distribution of renal osteodystrophy pattern of the first bone biopsy. (**a**) Whole population; (**b**) according to the K/DOQI PTH ranges. HT, high-turnover bone disease; LT, low-turnover bone disease; NL, normal histology.

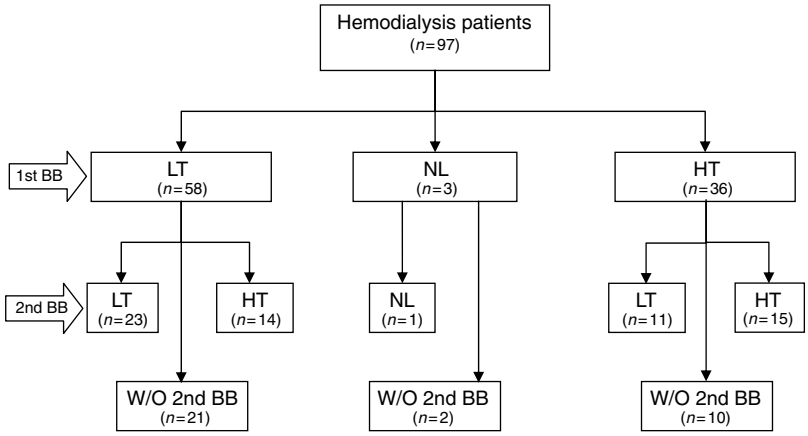

Figure 2 | **Flow diagram of bone disease types.**

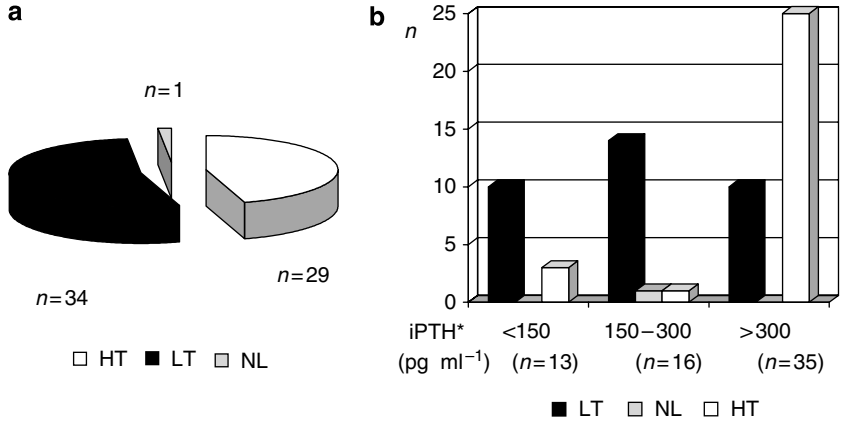

Figure 3 | **Distribution of renal osteodystrophy pattern of the second bone biopsy.** (**a**) Whole population; (**b**) according to the K/DOQI PTH ranges. HT, high-turnover bone disease; LT, low-turnover bone disease; NL, normal histology. *The mean level of iPTH during the follow-up was used to classify patients into the K/DOQI PTH ranges.

patient had normal bone histology. Similar to the results of the first bone biopsy, the most prevalent bone disorders observed below the lower and above the upper limit of the iPTH range were LT ($n=10$, 73%) and HT ($n=25$, 71%), respectively. Once again, LT ($n=14$, 87%) was the most common finding within the recommended range of iPTH (Figure 3b). The first bone biopsy from these 16 patients whose serum iPTH remained in the K/DOQI target range

Table 2 | Demographic and biochemical characteristics of the patients with iPTH target range (150–300 pg ml^{-1}) at baseline and at the end of the study

	Baseline (1st bone biopsy)		Ending (2nd bone biopsy)	
	LT (*n*=14)	HT+NL (*n*=8)	LT (*n*=14)	HT+NL (*n*=2)
Age (years)	53 ± 14.2	46.7 ± 9.2	48 ± 9.6	51 ± 1.4
Gender (% male)	71	62	57	50
Race (% white)	78	37	57	50
Time on hemodialysis (months)	28.1 ± 22.4	31.5 ± 24	43.2 ± 16.7	44.5 ± 20.5
Diabetes mellitus (%)	28.5	50	21.4	0
Dialyzate [Ca^{2+}]=2.5 mequiv. l^{-1} (%)	57	50	64	50
Calcitriol dosage (μg day^{-1})[a]	0.01 ± 0.02	0.5 ± 1.0	0.01 ± 0.03	0
Phosphate binder (%)				
Calcium acetate	36[b]	50[b]	21.4	50
Sevelamer	64[b]	50[b]	78.6	50
Serum ionized calcium (mmol l^{-1})	1.27 ± 0.11	1.27 ± 0.1	1.23 ± 0.07	1.3 ± 0.05
Serum phosphorus (mg per 100 ml)	5.3 ± 1.1	5.6 ± 1.4	5.3 ± 1.5	6.6 ± 0.4
Serum iPTH (pg ml^{-1})	210 ± 38	212.2 ± 38.3	224. ± 44.1	194.5 ± 46.2
Al.S/BS > 25% (%)	57	37.5	50	50

Al.S/BS, aluminum bone surface; HT, high-turnover bone disease; iPTH, intact parathyroid hormone; LT, low-turnover bone disease; NL, normal.
[a]Mean dose of calcitriol at baseline or during the follow-up.
[b]Refers to the percentage of patients allocated to each group of phosphate binder at the beginning of the study.

Table 3 | Demographic and biochemical characteristics of the patients with iPTH higher than 300 pg ml^{-1} at baseline

	Baseline (1st bone biopsy)	
	LT (*n*=15)	HT (*n*=25)
Age (years)	49.7 ± 16.1	43.4 ± 12.7
Gender (% male)	80	64
Race (% white)	73	44
Time on hemodialysis (months)	38 ± 22*	53.2 ± 28.7
Diabetes mellitus (%)	20	4
Dialyzate [Ca^{2+}]=2.5 mequiv. l^{-1} (%)	33	12
Calcitriol dosage (μg day^{-1})[a]	0.06 ± 0.1*	0.26 ± 0.25
Serum ionized calcium (mmol l^{-1})	1.24 ± 0.09	1.26 ± 0.07
Serum phosphorus (mg per 100 ml)	6.9 ± 1.9	6.0 ± 1.5
Serum iPTH (pg ml^{-1})	694.2 ± 352.7	803.9 ± 355.3
Al.S/BS > 25% (%)	60	32

Al.S/BS, aluminum bone surface; HT, high-turnover bone disease; iPTH, intact parathyroid hormone; LT, low-turnover bone disease.
*$P<0.05$.
[a]Mean dose of calcitriol at baseline.

Table 4 | Prediction of bone disease based on K/DOQI iPTH cut-off

	Sensitivity	Specificity	Positive predictive value
LT (ABD and OM) cut-off < 150 pg ml^{-1}	0.50	0.85	0.83
HT (PHBD and MUO) cut-off > 300 pg ml^{-1}	0.69	0.75	0.62

ABD, adynamic bone disease; HT, high-turnover bone disease; iPTH, intact parathyroid hormone; LT, low-turnover bone disease; MUO, mixed uremic osteodystrophy; OM, osteomalacia; PHBD, predominant hyperparathyroid bone disease.

during the 12 months of observation had unveiled the following histological distribution: 11 LT, 4 HT, and 1 NL. Only one patient from LT group changed to HT. All patients from HT changed their bone histology to LT. The patient with normal bone histology remained within the same category.

Table 2 shows the demographic and biochemical characteristics of the patients within the ideal range of iPTH, at the baseline and after 1-year follow-up. There was no difference between LT and HT + NL groups regarding demographic and biochemical parameters.

Table 3 shows the demographic and biochemical characteristics of the patients with serum iPTH values above the upper limit of its target range (> 300 pg ml^{-1}) at the baseline. Compared to HT, LT patients presented less time on hemodialysis, higher phosphorus levels, and have been taking a lower dose of calcitriol. There was no difference regarding age, gender, presence of diabetes, percentage of patients on 2.5 mequiv. l^{-1} dialyzate calcium concentration, serum ionized calcium, iPTH levels, and the percentage of patients with aluminum intoxication between groups. A borderline association ($P=0.07$) between LT and white race was observed.

Table 4 shows the type of bone disease predicted from the iPTH level. Intact PTH level below 150 pg ml^{-1} presented a 50% sensitivity, 85% specificity, and 83% positive predictive value for the diagnosis of LT. In contrast, iPTH level greater than 300 pg ml^{-1} presented a 69% sensitivity, 75% specificity, and 62% positive predictive value for the diagnosis of HT. The positive predictive value of the ideal range of iPTH for the identification of normal bone histology could not be performed in this study owing to the low number of patients with this histological pattern.

DISCUSSION

This study provides a current evaluation of the effectiveness of the target range of iPTH (150–300 pg ml^{-1}), as recommended by the NKF K/DOQI guidelines, in preventing

abnormalities of bone remodeling in maintenance hemodialysis patients. Furthermore, we analyzed the predictive value of the iPTH below the lower and above the upper limit of its target range to identify, respectively, LT and HT in this population.

Only 2 out of 22 patients with iPTH levels between 150 and 300 pg ml^{-1} presented normal bone histology at baseline. LT was the most prevalent histological finding in those patients. Indeed, HT and LT were the most frequent bone disorder in patients with iPTH levels above 300 pg ml^{-1} and below 150 pg ml^{-1}, respectively. Thereafter, patients were followed up monthly during 1 year. They were treated to achieve the goals of the bone metabolism parameters proposed by the NKF K/DOQI guidelines.[8] Strikingly, at the time point of the second biopsy, LT remained the most prevalent bone biopsy finding in those patients who had been able to achieve the iPTH target range as defined by the K/DOQI guidelines. Souberbielle *et al.*[9] have shown that Immulite 2000 intact PTH assay exhibits a positive median bias of 37.8% compared with the Allegro assay, which means that the iPTH target range would be 212–410 pg ml^{-1} for the Immulite assay. Even if this bias was taken into account, the prevalence of LT within this adjusted range would be similar as compared to that evaluated by Immulite assay (data not shown).

Although we could observe an association between traditional factors,[10–12] such as aging, diabetes, white race, less time on hemodialysis and lower PTH levels, and LT in the entire population, a similar finding could not be observed in patients with iPTH levels between 150 and 300 pg ml^{-1}. This lack of association may be attributed to the low number of patients presenting that condition. These results suggest that K/DOQI target range of iPTH is not necessarily a safe harbor to bone. Furthermore, even for iPTH >300 pg ml^{-1}, LT was observed in one-third of the patients. Of note, both LT and HT patients did not differ in any aspect, but in time on hemodialysis and dose of calcitriol.

Our data are in contrast with previous studies that have indicated that optimal iPTH range in dialysis patients appeared to be 2–4 times the upper limit of its normal levels.[8,13] However, one should consider that most of these studies were conducted in the early 1990s and many paradigms have been changed since this period. The use of aluminum hydroxide in dialysis patients was abandoned. Vitamin D and calcium-based phosphate binders are now prescribed with more caution due to their harmful extra skeletal effects. Treatment goals for ROD management are stricter. Additionally, new drugs, such as sevelamer, have been used for the treatment of mineral metabolism derangement. Although the impact of these advances on bone is not completely known, they have certainly modified the bone response to the uremic environment.

The lower cut-off level of the iPTH range provided a positive predictive value of 83% for the diagnosis of LT. Only a limited number of patients with HT had iPTH <150 pg ml^{-1}. Conversely, a high proportion of patients with iPTH >300 pg ml^{-1} presented LT, based on bone biopsy. This explains the low (69%) positive predictive value of the upper cut-off level of the iPTH range for the diagnosis of HT. Recently, Gal-Moscovici and Popovtzer[14] have also observed the occurrence of LT in patients with high PTH levels. Different from their study, this study provided information on the prevalence of ROD pattern according to the K/DOQI PTH target range. In addition, the prospective design allowed us to notice that normalization of bone turnover is difficult to be achieved even in patients who meet the PTH target range during 1-year follow-up. Other reports have elegantly raised concerns about the reliability of iPTH measurement as an indicator of bone turnover in CKD patients.[9,15] Intact PTH assays measure both full-length 1-84 PTH and N-terminal truncated 7-84 PTH fragment.[16] These molecules exhibit distinct actions on bone. It has been proposed that 7-84 PTH fragment may play a role in skeletal resistance to PTH observed in stage 5 CKD.[17] Indeed, the assay used for measuring iPTH in this study, that is Immulite, presents—according to the study conducted by Souberbielle *et al.*[9]—a ratio of recovery of those both compounds <1, which indicates that the assay crossreacts with 7-84 PTH fragment less than with 1-84 PTH. Although more specific PTH assays (1-84 PTH) are currently available,[18] there is still controversy about the clinical advantage of those assays over the traditional one.[19]

The applicability of other biochemical markers of bone turnover in CKD patients, such as bone-specific alkaline phosphatase, osteocalcin, and collagen breakdown products, has been investigated.[20–22] Their routine use in daily clinical practice has not been established yet.[2,23] Meanwhile, PTH measurement remains as the main surrogate indicator of bone turnover in CKD patients. Further, despite its disadvantages, bone biopsy followed by histomorphometric analysis is undoubtedly the most conclusive method to diagnose ROD.

The presence of aluminum intoxication could be a limitation in this study. Despite not using aluminum-based phosphate binder and strictly controlling water quality in the dialysis centers involved in this study, aluminum intoxication (Al.S/BS $>25\%$) was present in 41% of the entire population. However, one should notice that the amount of aluminum bone surface was not that high (Al.S/BS $= 24.3 \pm 28.6\%$). Other aluminum exposure source that may be important in patients with CKD, such as aluminum kitchen utensils,[24] were not investigated in this study. Other limitation was the method used for diagnosing aluminum overload. It was assessed only by bone histomorphometry, and not by a deferoxamine test, which would be the ideal approach to exclude aluminum overload.[25] It has been reported that the effect of aluminum on bone would depend on the pattern and amount of aluminum exposure. Thus, heavy aluminum loads could lead to a mineralization defect and, consequently, osteomalacia. In contrast, a long-term low-level aluminum exposure, combined with other factors that reduce bone turnover, such as calcium-based phosphate binders, may

affect bone metabolism inducing a pattern of bone damage characterized by low bone turnover.[26] However, Ferreira *et al.* found a similar distribution of bone biopsy findings across their hemodialysis patients for circulating iPTH values in the low, normal, or high range, as defined by the K/DOQI guidelines, in the absence of evidence for aluminum intoxication (Ferreira *et al.* Effects of sevelamer and calcium carbonate on bone mineralization and turnover in chronic maintenance hemodialysis patients. XLIII ERA-EDTA Congress, Abstract). It should be reinforced that no correlation was found between aluminum intoxication and the presence of LT in the studied population. Finally, the low number of patients in the iPTH target range, especially at the end of the study, precluded more powerful results. This was a consequence of the longitudinal design of the study and we obviously could not control the number of patients distributed in each PTH range after the follow-up.

In summary, to the best of our knowledge, this is the only prospective study that investigates bone histology in stage 5 CKD patients with iPTH levels within the target range recommended by the NKF K/DOQI guidelines. LT was quite frequent in the recommended iPTH range. Thus, the so-called ideal range of iPTH does not seem to guarantee a healthy bone. Additionally, one might conclude that the upper cut-off of iPTH ($300\,pg\,ml^{-1}$) should be revised. The poor predictive value of PTH for bone turnover makes it impossible to guess what the turnover would be in an individual patient even though statistically there may be correlation between PTH and bone turnover in the population at large. It is worthy mentioning that nephrologists should be aware that oversuppressing PTH by overuse of active vitamin D analogs is potentially harmful and aggravates hyperphosphatemia, hypercalcemia, and, ultimately, vascular calcification. These findings clearly show the limitations of the current recommendations based on iPTH levels for the management of ROD in maintenance hemodialysis patients. Our results underline the importance of more studies investigating this intricate issue of ROD.

MATERIALS AND METHODS

Study design and patients

This prospective 1-year follow-up study was originally designed as a randomized controlled trial to compare the effects of two types of phosphate binders, calcium acetate (PhosLo) and sevelamer (Renagel), on mineral metabolism, bone turnover, and progression of coronary calcification in patients undergoing hemodialysis.[27] Most importantly, all patients were older than 18 years, on hemodialysis for at least 3 months, had intact PTH levels below $1000\,pg\,ml^{-1}$, and should present hyperphosphatemia (serum phosphorus >5.5 mg per 100 ml) after a 2-week phosphate binder washout period. None of them had been previously submitted to parathyroidectomy. Indeed, besides calcium-based phosphate binder and calcitriol, none of them were using drugs that could interfere in bone metabolism, such as calcimimetics, corticosteroids, bisphosphonates, or hormonal replacement therapy. After the 2-week washout period, patients were randomized to receive either calcium acetate or sevelamer and were followed up for 1 year. Patients were submitted to a bone biopsy at baseline and at the end of the study. Ionized calcium and phosphorus were measured monthly and iPTH was measured every 2 months. Investigators were encouraged to adjust the dose of phosphate binder agent and calcitriol as well as the dialysate calcium concentration to achieve the targets proposed by the NKF K/DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease.[8] The target range for ionized calcium was that of the reference values of the method. Aluminum intoxication was treated with deferoxamine 5 mg per kg per week, IV, infused after the end of the first hemodialysis session of the week, during 6 months.

This study was conducted in four different dialysis centers, all of them located in the city of São Paulo, Brazil. All patients provided written informed consent. Local ethics committees approved the study protocol.

Biochemical parameters

Blood samples for the determination of biochemical parameters were obtained after an overnight fast and before the first weekly hemodialysis session. Serum ionized calcium (reference range: 1.11–1.40 $mmol\,l^{-1}$) and serum phosphorus (reference range: 2.3–4.5 mg per 100 ml) were determined by automated methods. Serum iPTH was measured by chemiluminescence assay (Immulite, DPC, Los Angeles, CA, USA; reference range: 10–65 $pg\,ml^{-1}$). During the follow-up, the mean serum level of iPTH was used to classify the patients into the K/DOQI PTH ranges at the end of the study.

Bone biopsy

Each patient was submitted to two transiliac bone biopsies, one at the entry and the other at the end of the study, using a standard technique described previously.[28] Bone specimens were obtained from alternate sides to avoid interference of repair processes at the site of the previous biopsy. Transiliac bone specimens were obtained with a 7-mm Bordier trephine after a course of double-labeling tetracycline ($20\,mg\,kg^{-1}\,day^{-1}$) for 3 days, separated by an interval of 10 days. The biopsies were performed 3–5 days after the last dose of tetracycline and no longer than 1 month after the blood sampling. After standard processing for histological studies,[29] undecalcified sections of trabecular bone were stained by toluidine blue (pH 6.4) and acid solochrome azurine for aluminum.[30] Unstained sections were used for tetracycline fluorescence analysis.

Bone histomorphometric analysis was conducted in a double-blind protocol, using the semiautomatic method provided by Osteomeasure software (Osteometrics Inc., Atlanta, GA, USA). All the histomorphometric parameters used were those suggested by the American Society of Bone and Mineral Research Histomorphometry Nomenclature Committee.[31] The reference ranges used for static parameters were obtained from local controls, whereas the dynamic parameters followed those described elsewhere.[32]

ROD was classified into one of the classical types according to the following criteria: (1) PHBD, defined as bone formation rate (BFR/BS; reference range: $0.13 \pm 0.07\,\mu m^3\,\mu m^{-2}\,day^{-1}$), as well as either osteoblast surface (Ob.S/BS; reference range: $2.13 \pm 3.8\%$) or osteoclast surface (Oc.S/BS; reference range: $0.07 \pm 0.22\%$), more than 1 s.d. above the normal range, osteoid volume (OV/BV; reference range: $3.2 \pm 3.0\%$) within or above the normal range and marrow fibrosis $>0.5\%$; (2) ABD, defined as BFR/BS and OV/BV more than 1 s.d. below the normal range and marrow fibrosis $<0.5\%$; (3) osteomalacia, defined as BFR/BS more than 1 s.d. below

the normal range and OV/BV more than 1 s.d. above the normal range; and (4) MUO, defined as BFR/BS, OV/BV, and mineralization lag time (reference range: 21.3 ± 2.3 days) more than 1 s.d. above the normal range and marrow fibrosis $>0.5\%$. Thereafter, these types were grouped into one of the two major patterns: HT (PHBD and MUO) or LT (osteomalacia and ABD). Aluminum intoxication was defined when aluminum bone surface (Al.S/BS) was greater than 25%.

Statistical analysis

Results are expressed as mean $\pm$ s.d. or percentages. Unpaired *t*-test was performed to compare continuous variables, and χ^2-test or Fisher's exact test for binary variables. The predictive value of the iPTH range for the diagnosis of the bone histological lesion was expressed in terms of sensitivity, specificity, and positive predictive value. $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 13.0 for Windows software.

DISCLOSURE

Genzyme Corporation provided the funding for this trial. The investigators were solely responsible for the design, conduct, analysis, and publication of the trial. There were no restrictions on publications, and all data were maintained and analyzed solely by the authors.

REFERENCES

1. Moe S, Druke T, Cunningham J *et al.* Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006; **69**: 1945–1953.
2. Martin K, Olgaard K. Diagnosis, assessment and treatment of bone turnover abnormalities in renal osteodystrophy. *Am J Kidney Dis* 2004; **43**: 558–565.
3. Andress DL, Endres DB, Maloney NA *et al.* Comparison of parathyroid hormone assays with bone histomorphometry in renal osteodystrophy. *J Clin Endocrinol Metab* 1986; **63**: 1163–1169.
4. Qi Q, Monier-Faugere MC, Geng Z, Malluche HH. Predictive value of serum parathyroid hormone levels for bone turnover in patients on chronic maintenance dialysis. *Am J Kidney Dis* 1995; **26**: 622–631.
5. Torres A, Lorenzo V, Hernandez D *et al.* Bone disease in predialysis, hemodialysis, and CAPD patients: evidence of a better bone response to PTH. *Kidney Int* 1995; **47**: 1434–1442.
6. Solal ME, Sebert JL, Boudailliez B *et al.* Comparison of intact, midregion, and carboxy terminal assays of parathyroid hormone for the diagnosis of bone disease in hemodialyzed patients. *J Clin Endocrinol Metab* 1991; **73**: 516–524.
7. Wang M, Hercz G, Sherrard DJ *et al.* Relationship between intact 1-84 parathyroid hormone and bone histomorphometric parameters in dialysis patients without aluminum toxicity. *Am J Kidney Dis* 1995; **26**: 836–844.
8. Eknoyan G, Levin A, Levin NW. National Kidney Foundation. Bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2003; **42**(Suppl 3): 1–201.
9. Souberbielle JC, Boutten A, Carlier MC *et al.* Inter-method variability in PTH measurement: implication for the care of CKD patients. *Kidney Int* 2006; **70**: 345–350.
10. Hercz G, Pei Y, Greenwood C *et al.* Aplastic osteodystrophy without aluminum: the role of 'suppressed' parathyroid function. *Kidney Int* 1993; **44**: 860–866.
11. Sherrard DJ, Hercz G, Pei Y *et al.* The spectrum of bone disease in end-stage renal failure—an evolving disorder. *Kidney Int* 1993; **43**: 436–442.
12. Gupta A, Kallenbach LR, Zasuwa G, Divine GW. Race is a major determinant of secondary hyperparathyroidism in uremic patients. *J Am Soc Nephrol* 2000; **11**: 330–334.
13. Felsenfeld AJ. Considerations for the treatment of secondary hyperparathyroidism in renal failure. *J Am Soc Nephrol* 1997; **8**: 993–1004.
14. Gal-Moscovici A, Popovtzer MM. New worldwide trends in presentation of renal osteodystrophy and its relationship to parathyroid hormone levels. *Clin Nephrol* 2005; **63**: 284–289.
15. Lepage R, Roy L, Brossard JH *et al.* A non-(1-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples. *Clin Chem* 1998; **44**: 805–809.
16. Reichel H, Esser A, Roth HJ, Schmidt-Gayk H. Influence of PTH assay methodology on differential diagnosis of renal bone disease. *Nephrol Dial Transplant* 2003; **18**: 759–768.
17. Slatopolsky E, Finch J, Clay P *et al.* A novel mechanism for skeletal resistance in uremia. *Kidney Int* 2000; **58**: 753–761.
18. Gao P, Scheibel S, D'Amour P *et al.* Development of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone 1-84: implications for improvement of accurate assessment of parathyroid function. *J Bone Miner Res* 2001; **16**: 605–614.
19. Lehmann G, Stein G, Huller M *et al.* Specific measurement of PTH (1-84) in various forms of renal osteodystrophy (ROD) as assessed by bone histomorphometry. *Kidney Int* 2005; **68**: 1206–1214.
20. Urena P, Hruby M, Ferreira A *et al.* Plasma total versus bone alkaline phosphatase as markers of bone turnover in hemodialysis patients. *J Am Soc Nephrol* 1996; **7**: 506–512.
21. Morishita T, Nomura M, Hanaoka M *et al.* A new assay method that detects only intact osteocalcin. Two-step non-invasive diagnosis to predict adynamic bone disease in haemodialysed patients. *Nephrol Dial Transplant* 2000; **15**: 659–667.
22. Urena P, Ferreira A, Kung VT *et al.* Serum pyridinoline as a specific marker of collagen breakdown and bone metabolism in hemodialysis patients. *J Bone Miner Res* 1995; **10**: 932–939.
23. Urena P, De Vernejoul MC. Circulating biochemical markers of bone remodeling in uremic patients. *Kidney Int* 1999; **55**: 2141–2156.
24. Lin JL, Yang YJ, Yang SS, Leu ML. Aluminum utensils contribute to aluminum accumulation in patients with renal disease. *Am J Kidney Dis* 1997; **30**: 653–658.
25. D'Haese PC, Couttenye MM, Goodman WG *et al.* Use of the low-dose desferrioxamine test to diagnose and differentiate between patients with aluminium related bone disease, increased risk for aluminium toxicity, or aluminium overload. *Nephrol Dial Transplant* 1995; **10**: 1874–1884.
26. Cannata-Andia JB. Reconsidering the importance of long-term low-level aluminum exposure in renal failure patients. *Semin Dial* 2001; **14**: 5–7.
27. Barreto DV, Barreto FC, Carvalho AB *et al.* Coronary calcification in hemodialysis patients: the contribution of traditional and uremia-related risk factors. *Kidney Int* 2005; **67**: 1576–1582.
28. Trueba D, Sawaya BP, Mawad H, Malluche HH. Bone biopsy: indications, techniques, and complications. *Semin Dial* 2003; **16**: 341–345.
29. Malluche HH, Faugere MC. *Methodology of Mineralized Bone Histology, in Atlas of Mineralized Bone Histology*. Karger AG, Basel: Switzerland, 1986, pp 17–24.
30. Dos Reis LM, Batalha JR, Muñoz R *et al.* Brazilian normal static bone histomorphometry: effects of age, gender and race. *J Bone Miner Metab* 2007; **25**: 400–406.
31. Parfitt AM, Drezner MK, Glorieux FH *et al.* Bone histomorphometry: standardization of nomenclature, symbols and units. *J Bone Min Res* 1987; **2**: 595–610.
32. Sherrard DJ, Baylink DJ, Wergedal JE, Maloney NA. Quantitative histological studies on the pathogenesis of uremic bone disease. *J Clin Endocrinol Metab* 1974; **39**: 119–135.